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ET 1202 (2005) (English): Honey - Specification

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ETHIOPIAN STANDARD

ES 1202:2005

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Honey - Specification

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Foreword

This Ethiopian Standard has been prepared under the direction of the Agriculture and Food Technology Technical Committee and published by the Quality and Standards Authority of Ethiopia (QSAE).

In preparing this Ethiopian Standard references have been made to the following :

-Indian Standard 4941-1974 specification for extracted honey (First revision)

-Codex Standard for honey - Codex standard 12-1981 Rev. (1987)

Acknowledgement has been made for the use of information from the above publication.

Honey - Specification

1 Scope

This Ethiopian Standard specifies requirements, method of sampling and test for honey.

2. Normative reference

The following Ethiopian Standard contain provisions, which through reference to this text constitute provisions of this Ethiopian Standard. At the time of publication the editions indicated were valid. All standards are subject to revision, and possibility of applying the most recent editions of the Ethiopian Standard indicated below. Registers of currently valid standards are maintained in the Quality and Standards Authority of Ethiopia.

ES 359:2001 General Standard for the labeling of pre packaged foods.

ES 261:2001 Drinking water - specification.

3 Definitions

For the purpose of this Draft Ethiopian Standard the following definitions shall apply.

3.1
honey
shall mean the natural sweet substance produced by honey bees from the nectar of blossoms which honey bee collect transform and combine with specific substance of the their own store and leave in the honey comb to ripen and mature.

3.2
nectar or blossom honey
shall mean honey, which is produced from nectarines of flowers.

3.3
extracted honey
shall mean honey obtained by centrifuging decapped brood less combs.

3.4
pressed honey
shall mean honey obtained by pressing brood less combs with or without the application of moderate heat.

3.5
crystallized or granulated honey
shall mean honey which has a fine crystalline structure and which may have undergone a natural process of solidification as a result of glucose crystallization

4.1 General

4.1.1 Honey shall not have any objectionable flavor, aroma, or taint absorbed from foreign matter during its processing and storage.

4.1.2 Honey shall not have begun to ferment or effervesce.

4.1.3 Honey shall not be heated to such an extent that its essential composition and quality are impaired.

4.1.3 Pressed honey shall not contain water insoluble solids more than 0.5% by mass.

4.1.4 Extracted honey shall not contain water insoluble solids more than 0.1% by mass.

4.1.5 The color of honey shall be uniform throughout and may vary from light to dark brown.

4.1.6 Honey shall not be heated to such an extent that its essential composition and quality is impaired.

4.1.7 Honey shall not contain any food additives, such as color, vitamins and minerals.

4.1.8 Honey shall be graded into three based on moisture content when determined according to Annex A

Grade A 17.5-19
Grade B 19.1-20
Grade C 20.1-21

4.1.9 Honey shall also comply with the requirements given in the following table.

Table I showing requirements for honey

Ser.No	Characteristics	Requirements	Test method
1	Apparent reducing sugar (as invert sugar), % by mass min.	65	Annex B
2	Apparent sucrose content % by mass, max.	10	Annex C
3	Water insoluble solids contents % by mass max.	0.1	Annex D
4	Mineral content (ash), % by mass max.	0.6	Annex E
5	Acidity mill equivalents acid per kg	40	Annex F
6	Diastase activity, 1% starch solution hydrolyzed by the enzyme in 1 gram of honey in 1 hour at $40^{o}C$, min.	3	Annex G
7	Hydroxymethyl furfural content mg/kg	40	Annex H

5. Hygiene

5.1 Honey shall be free form visible mould and as far as practicable, be free from inorganic or organic matters foreign to its composition, such as, insects insect debris, brood or grains of sand, when honey is used in any product for human consumption.

5.2 Honey shall not contain toxic substances arising from microorganisms or plants in an amount, which may constitute a hazard to health.

5.3 All surfaces hands and containers coming in to contact with the honey shall be clean.

5.4 Use of old and dark combs or blood combs which contribute to honey of darker colour, comb odour, higher acidity and faster aging shall be avoided.

5.5 In appropriate use of antibiotics and other drugs, chemicals to treat or prevent honeybee disease or pest control shall be avoided.

5.6 Location of hives in densely urbanized or industrialized zones or areas otherwise subjected to strong environmental pollution shall not be used.

5.7 Combs with residual honey from previous year and honey high in yeasts that result in faster fermentation and premature crystallization shall not be used.

5.8 If honey processed outside, processing shall not be done during a windy or rainy day.

5.9 Containers and processing equipment shall be made of materials compatible with honey. No copper, iron, steel or zinc shall be used as they dissolve into the honey and may affect colour and flavor, and might reach toxic levels.

5.10 Storage containers made of improper material shall be coated completely with beeswax or food grade plastic lines to avoid any direct contact.

5.11 Equipment and containers coming in contact with honey shall be designed and constructed to ensure that, where necessary shall be adequately cleaned, disinfected and maintained to avoid the contamination of honey.

5.12 Honey shall be extracted only form sealed combs. Carbolic acid or excessive smoke or toxic fumes shall not be used as it may taint the flavor of honey or other wise spoil it.

5.13 The extraction room or space shall be exceedingly clean as well as the space where the honeycombs are stored prior to processing. Extraction shall be done only in clean, fly proof enclosure. This will avoid bees visiting the extracted honey and drawing in it.

5.14 Maximum care shall be taken to protect honey from biological or chemical contamination.

5.15 Containers for cause, by-products and in edible substances, shall be specifically identifiable, suitably constructed and where appropriate, made of impervious material.

5.16 An adequate supply of potable water complying with ES 261:2001 with appropriate facilities for storage, distribution in processing area shall be available whenever necessary to ensure the safety and quality of honey.

5.17 Adequate drainage and waste disposal system and facilities shall be provided and designed and constructed so that the risk of contaminating honey or the potable water supply is avoided.

5.18 Adequate facilities, suitably designated, shall be provided for cleaning honey utensils and equipment. Such facilities shall have an adequate supply of hot and cold potable water where appropriate.

5.19 Personnel hygiene facilities shall be available to ensure that an appropriate degree of personal hygiene can be maintained and to avoid contaminating honey where appropriate, facilities shall include;

a) Adequate means of hygienically washing and drying hands, including washbasins and supply of water.

b) Lavatories of appropriate hygienic design and adequate changing facilities for personnel.

5.20 Honey handlers shall maintain a high degree of personal cleanliness and where appropriate wear suitable protective clothing, head covering, and footwear cuts and wounds where personnel are permitted to continue working, shall be covered by suitable water proof dressings.

5.21 Personnel shall wash always their hands, at the start of honey handling activities, immediately after using the toilet; and after handling any contaminated material.

5.22 People engaged in honey handling activities shall refrain from behavior such as, smoking, spitting, chewing or eating, sneezing or coughing over unprotected honey, which could result in contamination.

5.23 Packing design and material shall provide adequate protection for honey to minimize contamination, prevent damage, and accommodate proper labeling.

5.24 Storage containers for liquid or crystallized honey shall be made either of glass or stainless or coated with food approved plastic paint or beeswax.

5.25 Nothing shall be allowed to impart any odor to honey during storage.

5.26 If recycled container are used care shall be taken that they are absolutely clean and have not the slightest residual odour.

5.27 Containers previously used for toxic chemicals, oils or petroleum products shall never be used for storing any bee products even after coating within paint plastic or bees wax.

5.28 Opening in wholesale containers shall have to be big enough to facilitate removal of crystallized honey.

5.29 Containers of honey lid shall be airtight and kept away from heat and light. Moreover most products containing honey shall be protected from excessive moisture by special packing.

5.30 Storage rooms shall have a temperature near 20°C and a relative humidity of less than 65% storage of honey at more than 25°C causes increasing quality less with time; due to progressive chemical and enzymatic changes. Proper storage and packing together with quick marketing and consumption will reduce or eliminate the need for preservatives.

5.31 Honey shall be adequately protected during transportations.

5.32 Where necessary, conveyances and containers shall be designed so that they do not contaminate honey or packaging.

a) Permit effective separation of different foods or honey from non-food items where necessary during transport.

b) Provide effective protection from contamination including dust and fumes.

6 Packing and labeling

6.1 Packing

6.1.1 Honey shall be packed in hygienically clean, wide mouthed, glass container or in acid - resistant lacquered tin container or in suitable polyethylene containers. The screwed caps of the glass containers shall be of non-corrosive and non-reactive material to honey and shall be provided with cork washers to avoid spilling.

6.1.2 Packing materials shall not impart any odour to the honey. Particularly if used containers are recycled, care shall be taken that they are absolutely clean and have not the slightest residual odor.

6.2 Labeling

6.2.1 Honey shall be labeled in accordance with ES 359: 2001 General Standard for the labeling of pre-packaged foods and shall contain additionally the following information.

i) Name of the material

ii) Name of the packer

iii) Batch or code number

iv) Net weight

v) Date of packing

vi) Expiry date

7. Sampling

7.1 General requirements

7.1.1 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

7.1.2 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

7.1.3 The sampling instrument shall be clean and dry when used.

7.1.4 Precautions shall be taken to protect the samples the material being sampled the sampling instrument and the containers for samples from adventitious contamination.

7.1.5 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely field by the sample.

7.1.6 Each container shall be sealed air-tight after filling and marked with full detail of sampling code number and other important particulars of the consignment.

7.1.7 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

7.2 Scale of Sampling

7.2.1 Lot. All the containers in a single consignment belonging to the same material shall constitute a lot. If the consignment is declared to consist of different grades of material the containers belonging to the same grade shall be grouped together and the groups of containers of the same grade in a consignment shall constitute separate lot.

7.2.2 Sample shall be tested from each lot for ascertaining its conformity to the requirements of the specification in section 4.1.9.

7.2.3 The number of containers to be sampled form each lot shall depend on the size of the lot and it shall be done according to the following table.

Table II showing number of containers to be selected for sampling.

Lot size	Number of containers to be selected	
	500 g. or above	Below 500 g.
Up to 25	3	5
26 to 150	4	6
151 to 500	5	9
501 and above	7	12

7.2.4 The containers shall be selected at random from the lot.

7.3 Preparation of individual sample

7.3.1 Draw with suitable sampling instrument equal quantities of the material from different parts, top, middle, bottom of the container till about 300 g. of the material is drawn divide it into three equal parts.

7.3.2 Each part so obtained shall constitute an individual sample representing the container and shall be transferred immediately to thoroughly clean and dry container and sealed air-light, and marked with all necessary information.

7.3.3 The individual samples so obtained from each container shall be made into sets in such a way that each set has a sample representing each selected container. One of these shall be marked for the supplier, another for the laboratory and the third for the referee.

7.4 Preparation of Composite Sample

7.4.1 From the material from each of the selected container, remaining after the individual sample has been taken approximately equal quantities of the material shall be taken and mixed together so as to form a composite sample weighing bout 150g. This composite sample shall be divided into three equal parts and transferred to clean and dry containers selected airtight and labeled with all the necessary information. One of these composite samples shall be for the supplier, another for the laboratory and the third for the referee.

7.4.2 When honey is in containers of size less than 500 g the number of the containers shall be selected according to column 3 of table 2. The selected containers shall be divided at random into three equal sets. The containers belonging to each set shall be opened and approximately equal quantity of material shall be taken and mixed together to form a composite sample of 50 g. The honey left in each container after the preparation of composite sample shall be sealed air-tight and labeled with all the necessary information.

7.4.3 The individual samples also shall be packed in air-tight containers with all the necessary information. The three sets of the individual samples with their corresponding composite sample shall be marked in such a way that one set is for the supplier, another for the laboratory and the third for the referee.

7.5 Referee Sample

7.5.3 Referee Samples shall consist of a set of individual samples and a composite sample marked for this purpose and shall bear if possible seals of the supplier and signature of the sampler. These shall be kept at a place at the appropriate place.

7.6 Number of tests

7.6.3 Tests for moisture, ash, total reducing sugars, HMF and diastase activity shall be conducted on each of the samples constituting a set of individual samples.

7.6.4 The test for sucrose percent, fructose-glucose ratio and acidity shall be conducted on the composite sample.

7.7 Criterion for conformity.

7.7.1 A lot shall be declared to have satisfied the requirements of the specification specified in section 4

Each individual sample shall satisfy the requirements given in Section &4.1.9

Annex A
(normative)

Determination of Moisture Content

A1 Principle

Refractive index of the sample is read and the value is converted to moisture content from a table

A2 Apparatus

A2.1 Refractometer

A3 Procedure

A3.1 Determination of the Refractive Index

Determine the refractive index of the test sample using a refractometer at a constant temperature near 20 ^{0}C.

A3.2 Convert the reading to moisture content (percent by mass) using the table given below. If the determination is made at a temperature other than 20 ^{0}C, convert the reading to standard temperature of 20 ^{0}C, according to the temperature corrections quoted. The method used shall be noted in the test report.

Table I showing for the estimation of moisture content

Refractive index (20 ^{0}C)	Moisture content (Percent)	Refractive index (20 ^{0}C)	Moisture content (Percent)	Refractive index (20 ^{0}C)	Moisture content (Percent)
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.6
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	25.0
1.4951	16.6	1.4845	20.8	1.4740	25.8
1.4946	16.8	1.4840	21.0		
1.4940	17.0	1.4835	21.2		

Annex B
(normative)

Determination of reducing sugar content

B1 Principle

B1.1 The method is a modification of the Lane and Eynon procedure involving the reduction of Soxhlet's modification of Fehlng's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator.

B1.2 The maximum accuracy for this type of determination is attained by ensuring that the reduction of the Fehling's solution during the standardization step and in the determination of the reducing sugars in the honey solution are carried out at constant volume. A preliminary titration is, therefore, essential to determine the volume of water to be added before the determinations are carried out to satisfy this requirement.

B2 Reagents

B2.1 Soxhlet's modification of Fehling's solution

B2.2 Solution A: Dissolve 34.64 g copper sulphate pent hydrate ($CuSO_4.5H_20$) with distilled water to 500 ml. Keep one day before titration.

B2.3 Solution B: Dissolve 173 g sodium potassium tartarate (C_4H_4 K $Na0_6.4H_20$) and 50g sodium hydroxide (NaOH) with distilled water to 500ml. Filter through inert material.

B2.4 Standard Invert Sugar Solution (10 g/L)

B2.4 .1 Weigh accurately 9.5 g pure sucrose, add 5 ml hydrochloric acid ca. 36.5 percent w/w pure HCl) and dilute with water to about 100 ml, store this acidified solution for several days at room temperature (ca. 7 days at 12^{0}Cto 15^{0}C,or 3 days at 20^{o}C to 25 ^{o}C), and then dilute to I liter.

(N.B. Acidified 1.0 percent invert sugar remains stable for several months). Neutralize a suitable volume of this solution with IM sodium hydroxide solution (40 g/l) immediately before use and dilute to the required concentration (2 g/l) for the standardization

B2.5 methylene Blue Solution. Dissolve 2g in distilled water and dilute to 1 litter.

B2.6 Alumina Cream

B2.6.1 Prepare cold saturated solution of alum ($K_2S0_4A1_2$ $(S0_4)_3$.24H20) in water. Add ammonium hydroxide with constant

stirring until solution is alkaline to litmus, let precipitate settle and wash by decantation with water until wash-water gives only slight test for sulphate with barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

B3 Procedure

B3.1 sample preparation

B3.1.1 Liquid or Strained Honey

If sample is free from granulation, mix thoroughly by stirring or shaking; if granulated, place closed container in water-bath without submerging, and heat 30 minutes. at 60 ^{o}C; then if necessary heat at 65 ^{o}C until liquefied. Occasional slaking is essential. Mix thoroughly and coal rapidly as soon as sample liquefies. Do not heat honey intended for hydroxy methyl furfural or diastatic determination. if foreign matter, such a s wax, sticks, bees, particles of comb, etc is present, heat sample to 40 ^{0}C in water-bath and strain through cheesecloth in hot-water-funnel before sampling..

B3.1.2 Preparation of test Sample (applicable to honeys which may contain sediment)

(a) Transfer an accurately weighed sample of approximately 25 g (W1) from the homogenized honey to 100 ml volumetric flask add 5ml alumina cream dilute to volume with water at 20 ^{0}C and filter.

(b) Dilute 10 ml of this solution to 500 ml with distilled water (diluted honey solution).

OR

Weight accurately a representative quantity of about 2 g (W2) of the homogeneous honey sample, dissolve in distilled water and dilute to 200 ml- in a volumetric flask (honey solution).

(c) Dilute 50 ml of the honey solution to 100 ml Using distilled water (diluted honey solution).

B3.2 Standardization of the modified Felhling's solution

B3.2.1 Standardize the modified Felhling's solution A so that exactly 5 ml (pipette), when mixed with approximately 5 ml of Fehling's solution B, will react completely with 0.050 g invert sugar added as 25 ml dilute invert sugar solution (2 g/l).

B3.2.1.1 Preliminary Titration

The total volume of the added reactants at the completion of the reduction titration must be 35 ml. This is made up by the addition of a suitable volume of' water before the titration commences. Since the compositional criteria of the honey standard specify that there should be more than 60 percent reducing sugars (calculated as invert sugar) a preliminary titration is necessary to establish the volume of water to be added to a given sample to ensure the reduction is carried out at constant volume. This volume of water to be added is calculated by subtracting the volume of diluted honey solution consumed in the preliminary titration (C ml) from 25 ml.

Pipette 5 ml Fehling's solution A into a 250 ml Erlenmeyer flask and add approximately 5 ml**.** Fehling's Solution B. Add 7 ml, distilled water a little powdered pumice or other suitable antibumping agent, followed by about 15 ml diluted honey solution from a burette. Heat the cold mixture by boiling over wire gauze, and maintain moderate ebullition for 2 minutes. Add 1 ml 0.2 percent aqueous methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes, by repeated small additions of diluted honey solution until the indicator is decolorized. It is the colour of the supernatant liquid that must be observed. Note the total volume of diluted honey solution used (X rnl).

B4. Determination

B4.1 Calculate the amount of added water necessary to bring the total volume of the reactants at the completion of the titration to 35 ml by subtracting the preliminary titration (X ml) from 25 ml

B4 .2 Pipette 5 ml Fehling's solution A into a 250 ml Erlenmeyer flask and add approximately 5 ml Fehling's solution B

B4 .3 Add (25-X) ml distilled water, a little powdered pumice or other suitable anti bumping agent and, from a burette, all but 1.5 ml of the diluted honey solution volume determined in the preliminary titration. Heat the cold mixture to boiling over wire gauze and maintain moderate ebullition for 2 minutes. Add 1.0 rnl 0.2 percent methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes. By repeated small additions of diluted honey solution until the indicator is decolorized. Note the total volume of diluted honey solution (Y ml). Duplicate titration should agree within 0.1 rnl.

B5.1 Calculation and expression of results

Where the first procedure (B3.1.2,) has been used:

$$C=\frac{25}{W1}X\frac{1000}{Y1}$$

Where the second procedure has been used:

$$C=\frac{2}{W2}X\frac{1000}{Y2}$$

Where C = g invert sugar per 100 g honey

W1= weight (g) of honey sample taken according to the first procedure

W2 = weight (g) of honey sample taken according to second procedure

Y1= volume (ml) of diluted honey solution consumed in the determination carried out according to the first procedure

Y2= volume (ml of diluted honey solution consumed in the determination carried out according to the Second Procedure

Notes on the procedure

It is essential to the accuracy and repeatability of the determination that the volume of water necessary to bring the reactant mixture to a total volume of 35 ml be determined for each individual sample; the following table gives typical volumes which may be encountered at the preliminary titration stage for the incremental contents of invert sugar shown, assuming the test sample weighs about 25,g or test sample weighs about 2 g.

Table I showing volume of water to be added

Invert sugar content	Volume of distilled water to be added
%	ml
60	8.3
65	9.6
70	10.7
75	11.6

Annex C
(normative)

Determination of Apparent Sucrose Content

C1 Reagents

C1.1 Soxhlet modification of Felhling's solution

C1.2 Standard invert sugar solution

C1.3 Hydrochloric acid (6.34M aqueous)

C1.4 Sodium hydroxide solution 2g/l litre

C1.5 Methvlene blue solution 2 g/litre

Note all reagents are prepared as in annex B

C.2 Sample preparation

C2.1 Prepare the honey sample as in annex B

(a) Dilute 10 ml of this solution to 250 ml with distilled water honey solution (for sucrose determination)

OR

Prepare the honey solution as in annex B

C3 Procedure

C3.1 Hydrolysis of the test sample.

The honey solution (50 ml) is placed in a 100 ml volumetric flask, together with 25 ml distilled water; heat the test sample to 65 0C over a boiling water bath. The flask is then removed from the water-bath and 10 ml of 6.34 M hydrochloric acid added. The solution is allowed to cool naturally for 15 minutes, and then brought to 20 0C and neutralizing with 5M sodium hydroxide, using litmus paper as indicator, cooled again, and the volume adjusted to 100 ml (diluted honey solution).

C3.2 Titration

C3.2.1 As in annex B

C3.3 Calculation and expression of results

Calculate percent invert sugar (g invert sugar per 100 g honey) after inversion using the appropriate formula as percent invert sugar before inversion in annex B

Apparent sucrose content = (invert sugar content after inversion - invert sugar content before inversion) x0.95

The result is expressed as g apparent sucrose per 100 g honey.

Annex D
(normative)
Gravimetric determination of water-insoluble solids content

D1 Apparatus

D1.1 Balance

D1.2 Sintered glass pore size 4

D1.3 Oven maintained at 130 ^{0}C

D2 Procedure

D2.1 sample preparation

D2.1.1 The honey sample is prepared as in annex B.

D2.1.2 20 g of honey is weighed to the nearest 0.01g and dissolved in a suitable quantity of distilled water at 80 ^{0}C and mixed well.

D2.2 Gravimetric determination

D2.2.1 The test sample is filtered through a previously dried and weighed fine sintered glass crucible and washed thoroughly with hot water (80 ^{0}C) until free from sugar. The crucible is dried for one hour at 130 ^{0}C, cooled and weighed to the nearest 0.1 mg

D2.2.2 Expression of results

The result is expressed as percent water-insoluble solids (m/m).

Insoluble solids % by mass= $\frac{M1 - MX100}{W}$

Where,

M1=mass of the residue and the crucible

M= mass of the crucible

W= mass of the test portion

Annex E
(normative)

Determination of mineral content (ash)

E1 Apparatus

E1.1 Muffle furnace

E1.2 Platinium, Silica or porcelain crucible

E1.3 Bunsen burner or infrared lamp

E1.4. Desiccator

E2. Procedure

E2.1 Honey 5 to 10 grams is weighed accurately into an ignited and pre-weighed platinum, silica or porcelain crucible and gently heated in muffle furnace until the sample is black and dry and there is no danger of loss by foaming and overflowing. An infra-red lamp or Bunsen burner can also be used to char the sample before inserting into the furnace. If necessary, a few drops of

Olive oil may be added to prevent frothing. The sample is then ignited at 600 ^{0}C to constant weight. The sample is cooled in desiccator before weighing to constant weight.

$$\text{Ash, \%by mass} = \frac{W1 - W2}{M}$$

Where,

W1 =weight of empty crucible

W2 = weight of the ash and crucible

M = mass of the sample taken for the test

Annex F
(normative)

Determination of Acidity.

F1 Apparatus

F1.1 Balance

F1.2 Burette 10 ml

F2 Reagents

F2.1 Sodium hydroxide 0.1N (carbonate-free)

F2.2 Phenolphthalein indicator l percent (m/v) in ethanol, neutralized.

F2.3 Distilled Water made carbon dioxide free by boiling and subsequent cooling.

F3 Procedure

F3.1 Preparation of test sample

10.0 g of honey accurately weighed and dissolved in 75 ml distilled water

F3.2 Titration

The test sample is titrated against carbonate free 0.1 N sodium hydroxide solution using 4-5 drops of phenolphthalein indicator. The end-point Colour should persist for 10 seconds. For darkly colored samples, a smaller weight shall be taken. As an alternative, a pH meter may be used and the sample titrated to PH 8.3.

F3.3 Calculation and expression of results

in the neutralization The result is expressed as milli-equivalents acid/kg honey and is calculated as follows:

Acidity =10V

Where,

V = the volume of 0.1N NaOH used of 10 g honey.

Annex G
(normative)

Determination of Diastase Activity

G1 Reagents

G1.1 Iodine stock solution

Dissolve 8.8 g of iodine analytical grade, in 30-40 ml water containing 22 g potassium iodide, analytical grade, and dilute to I litre with water.

G1.2 Iodine solution 0.0007 N:

Dissolve 20 g potassium iodine, analytical grade, in 30-40 ml water in a 500-mL volumetric flask Add 5.0 ml iodine stock solution and make up to volume. Make up a fresh solution every second day.

G1.3 Acetate buffer - pH 5.3 (1.59.M)

Dissolve 87 g sodium acetate in 400 ml water, add about 10.5 ml glacial acetic acid in a little water and make up to 500 ml. Adjust the pH to 5. 3 with sodium acetate or acetic acid as necessary using a pH meter.

G1.4 Sodium Chloride Solution 0.5M:

Dissolve 14.5 g sodium chloride, analytical grade, in boiled-out distilled water and make up to 500ml The keeping time is limited by mould growth..

G1.5 Starch solution:

(a) Preparation of soluble starch

In a conical flask immersed in a water-bath and fitted with a reflux condenser, boil 20 g of potato starch for one hour in the presence of a n fixture of 100 ml of 95 percent ethanol and 7 ml of 1M hydrochloric acid. Cool, filter through a filtering crucible (pore size 90 - 150 -µm) and wash with water until the wash/water ceases to give any chloride reaction. Drain thoroughly and dry the starch in air at 35 ^{0}C the soluble starch must be stored in a well stoppered flask.

(b) Determination of moisture content of soluble starch

Accurately weigh a quantity of approximately 2 g of soluble starch and spread in a thin layer over the bottom of a weighing bottle. Dry for one and a half hours at 130 ^{0}C. Allow to cool in a dessicator and re-weigh. The weight loss with respect to 100 g represents the moisture content. The moisture content of such starch should be 7-8% m/m depending oil the humidity of the air in which the sample has been dried.

(c) Preparation of starch solution

Use a starch with a blue value between 0.5-0.55 using a I cm cell, as determined by the method below. Weight out the amount of starch, which is equivalent to 2.0 g anhydrous starch. Mix with 90 ml of water in a 250 ml conical flash. Bring rapidly to the boil, swirling the solution as much as possible, heating over a thick wire gauze preferably with an asbestos center. Boil gently for 3 minutes, cover and allow to cool spontaneously to room temperature. Transfer to a 100 ml volumetric flask, place in a water bath at 40 0C to attain this temperature and make up to volume at 40 0C

Method for determining blue value of starch

The amount of starch equivalent to 1g anhydrous starch is dissolved by the above method, cool and 2.5 acetate buffer added before making up to 100 ml in a volumetric flask.

To a 100 ml volumetric flask add 75 ml water, 1 ml 1M hydrochloric acid and I.5 ml of 0.02 N iodine solution. Then add 0.5 ml of the starch solution and make up to volume with water. Allow to stand for one hour in the dark and read in 1cm cell using a spectrophotometer at 660 nm against a blank containing all the ingredients except the starch solution.

Reading on the absorbance scale = Blue value.

G2 Apparatus

G2.1 Water-bath maintained at 40+ 0.2 ^{0}C.

G2.2 Spectrophotometer to read at 660 nm

G2.3 Test sample preparation

G2.3.1 The honey sample is prepared as in annex B without any heating.

G3 Procedure

G3.1 Honey solution: 10.0 g honey is weighed into a 50 ml beaker and 5.0 ml acetate buffer solution is added, together with 20 ml water to dissolve the sample. The sample is completely dissolved by stirring the cold solution. 3.0 ml sodium chloride solution is added to a 50 ml volumetric flask and the dissolved honey sample is transferred to this and the volume adjusted to 50 ml.

N.B: It is essential that the honey should be buffered before; coming into contact with sodium chloride.

Standardization of the starch solution

The starch solution is warmed to 40 0C and 5 ml pipetted into 10 ml of water at 40 0C and mixed well. 1 ml of this solution is pipetted into 10 ml 0.0007 N iodine solution, diluted with 35 ml of water and mixed well. The color is read at 660 nm against water blank using a 1 cm cell.

The absorbance should be 0.760 +0.020. If necessary the volume of added water is adjusted to obtain the correct absorbance.

G3.2 Absorbance determination

Pipette 10 ml honey solution into 50 ml graduated cylinder and place in 40 ^{0}C± 2 ^{0}C water-bath with flask containing starch solution. After 15 minutes, pipette 5 ml starch solution into the honey solution, mix, and start stop-watch. At 5 minutes intervals remove 1ml aliquots and add to 10.ml 0.0007N iodine solution. Mix and dilute to 50ml. Determine absorbance at 660 nm in spectrophotometer immediately using 1cm cell. Continue taking 1 ml aliquots at intervals until absorbance of less than 0.235 is reached.

G3.3 Calculation and expression of results

The absorbance is plotted against time (mm) on a rectilinear paper. A straight line is drawn through at least the last three points on the graph to determine the time when the reaction mixture reaches an absorbance of 0.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as ml 1

Percent starch solution hydrolyzed by the enzyme in 1 g of honey in 1 h at 40 ^{0}C. This diastase number corresponds with the Gothe-scale number,

Diastase activity = DN = ml starch solution one percent/g honey/h at 40 ^{0}C.

Annex H
(normative)

Hydroxymethylfurtural in Honey Spectrophotometric Method

H1 apparatus

H1.1 Spectrophotometer.UV to measure absorbance at 284 and 336 nm

H1.2 Analytical balance

H1.3 Filter paper

H1.4 Pipettes 5ml

H1.5 Test tubes 18x150mm

H1.6 Vortex mixer

H1.6 Beaker

H1.7 Volumetric flasks 50ml

H2 Reagents

H2.1 Carrez solution I Dissolve 15 g $K_4Fe(CN)_6.3H_20$ and dilute to 100 ml with distilled water.

H2.2 Carrez solution II -Dissolve 30 g Zn acitate and dilute to 100 ml with distilled water.

H2.3 Sodium bisulfate solution 0.20%. Dissolve 0.20 g $NaHSO_3$ and dilute to 100 ml with distilled water dilute 1+ 1 for reference if necessary Prepare fresh daily.

H3 procedure

H3.1 Accurately weigh 5 g honey in small beaker and transfer with total of 25 ml distilled water to 50 ml volumetric flask. Add 0.50 Carrez solution I, mix, add 0.50 ml Carrez solution II, mix, and dilute to volume with distilled water Drop of alcohol may be added to suppress foam. Filter through filter paper, discarding the first 10 ml filtrate.

H3.2 Pipette 5 ml filtrate into each of two 18 X 150 mm test tubes. Add 5.0 ml distilled water to one tube (sample) and 5.0 ml $NaHS0_3$ solution to other (reference). Mix well by using Vortex mixer and determine. Absorbance of sample against reference at 284 and 336 nm in 1 cm cells. If absorbance is greater than 0.6, dilute sample solution with water and reference solution with 0.1% $NaHS0_3$ solution to same extent and correct absorbance for dilution.

Mg Hydroxymethylfurfural (HMF)/100 g honey= $(A_{284} - A_{336})$ x 14.97 X 5/g sample